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Effects of dietary phosphorus and zinc levels on growth and bone mineralization in fingerlings of rainbow trout, *Oncorhynchus mykiss*

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Abstract.
A laboratory based 2 × 2 factorial experiment was conducted to investigate the influences of dietary phosphorus and zinc levels on growth and bone mineralization in fingerlings of rainbow trout for 21 weeks. Two levels of phosphorus (19 and 30 mg/g) and two levels of zinc (55 and 103 μg/g) in the dry diets were tested. Duplicate tanks of 30 rainbow trout (average weight 1.56 ± 0.24 g) per 60L glass tank were fed experimental diets three times a day to apparent satiation level at 15 to 24°C water temperature. The results of the present study demonstrated that dietary phosphorus supplementation influenced the growth and bone mineralization whereas zinc levels significantly ($p<0.05$) influenced bone mineralization in rainbow trout. Further investigations in this area with different size and age groups of this fish are broadly needed.

Key words: Phosphorus, Zinc, Bone mineralization, Rainbow trout

Introduction

Rainbow trout, which belongs to the family Salmonidae, is originally taxonomically linked with the Atlantic or Eurasian trouts of the genus *Salmo*. However, on the basis of evolutionary evidence which showed that rainbow trout has a greater genetic affinity to the Pacific salmon than to the Eurasian trouts so it was renamed as *Oncorhynchus mykiss* (Groot 1996). Rainbow trout have been introduced widely in suitable habitat throughout North America and other parts of the world, such as South America, Europe, Southern Asia, Japan, Africa and Oceania (Groot 1996, Scott and Crossman 1975).

Rainbow trout has important commercial and sport value. Their simple requirements for incubation under artificial conditions have made it possible to introduce to many new areas in the world. New understanding of the biological requirements of this fish and refinements of incubation and rearing methods have opened important possibilities for trout farming (Groot 1996). In the natural

environment rainbow trout feeds on various invertebrates including plankton, larger crustaceans, fish, insects, snails, and leeches. Nutritional requirements of rainbow trout have been well studied (NCR 1993). Their growth is very rapid when fed on well-balanced dry pelleted diets. So in many places around the world, they are entirely raised in artificial ponds on trout farms. Rainbow trout is by far the most widely farmed trout in the world and one of the few species of fish that may be regarded as truly domesticated. Trout production is predicted to increase by 5% per year for the near future and will likely maintain its place in the top 15 finfish and crustacean aquaculture species produced in the world, as well as remain in the top 10 species, with respect to total value (Hardy *et al.* 2000).

Dietary phosphorus (P) is an essential nutrient for optimum growth and metabolism of fish. It is the most important mineral needed by fish, since its requirement and functions are superior to that of any other mineral element (NCR 1993, Satoh *et al.* 2002). Zinc is also essential in the diet since waterborne Zn is not efficiently absorbed by aquatic animals as they required (NRC 1993). It is also known to be an essential trace element for growth (Burch *et al.* 1975, Semard 1999). Zinc is largely deposited in muscle, kidney, liver, pancreas, and bones (Wapnir 1990). Its deficiency may result in growth retardation (Ninh *et al.* 1995, 1996, Dorup *et al.* 1991). Among the tissues, bone has the highest Zn level and it serves as a reservoir for this element. In addition, Zn like IGF-1 can increase the protein component of bone and may play a role in bone growth (Ma and Yamaguchi 2001) whereas Zn deficiency resulted in lower levels of growth hormone and growth hormone binding protein mRNA (McNall *et al.* 1995).

Therefore, the present study aimed to investigate the possible effects of dietary P and Zn supplementation on the growth and bone mineralization of fingerling of rainbow trout using practical type diets.

Materials and methods

Formulation and composition of the experimental diets are presented in Table 1. Practical diets were formulated to contain 19 and 30 mg/g P, using monocalcium phosphate and 54 and 103 μg/g Zn, employing zinc sulfate heptahydrate (Table 1). The diets were labeled as P0Z0, P0Z1, P1Z0 and P1Z1 according to factors (P and Z) and levels (0 and 1). The experimental diets were formulated to be isocaloric and isonitrogenous. The carbohydrate sources and binders were wheat flour and pregelatinized starch, and the lipid source was pollock liver oil. The experiment was conducted in a 2 × 2 factorial design with the factors 'dietary phosphorus level 'and 'supplemental Zn level'.

The mineral mixture used in this study was the modified form of Ogino salt mixture (Ogino *et al.* 1979). The experimental diets were formulated to be isocaloric and isonitrogenous. The diets were pelleted using the laboratory pelletizer (AEZ12M, Hiraga-Seikakusho, Kobe, Japan), dried in a vacuum freeze-drier (RLE-206, Kyowa Vacuum Tech., Saitama, Japan), and stored at 4°C until used. The proximate

composition and mineral contents of the experimental diets used in this study are shown in Table 2 and Table 3. The diet was prepared with 57% (FM) as the sole protein source. Ingredients used in the test diets were selected taking into consideration the amino acid balance of the whole protein sources (Watanabe *et al.* 1993).

Table 1. Formulation and composition of the experimental diets

Ingredients (%)	Diets			
	P0Z0	P0Z1	P1Z0	P1Z1
Jack mackerel meal	57	57	57	57
Wheat flour	20	20	20	20
Pregelatinized starch	5	5	5	5
Pollock liver oil	4	4	4	4
Soybean oil	5	5	5	5
Mineral premixture[a]	0	1	0	1
Zn free mineral mixture[b]	1	0	1	0
Ca $(H_2PO_4)_2$	0	0	4	4
Vitamin premixture[c]	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5
Vitamin E (50%)	0.1	0.1	0.1	0.1
Cellulose	5.9	5.9	5.9	5.9

[a] Mineral premixture (%): NaCl 5.0, Mg $SO_4 \cdot 7H_2O$ 74.5, $FeC_6H_5O_7 \cdot nH_2O$ 12.5, Trace element mix.[a*] 5.0, Cellulose 3.0 [a*] (%)—$ZnSO_4 \cdot 7H_2O$ 35.3, $MnSO_4 \cdot 5H_2O$ 16.2, $CuSO_4 \cdot 5H_2O$ 3.1, $AlCl_3 \cdot 6H_2O$ 1.0, $CoCl \cdot 6H_2O$ 1, KIO_3 3, cellulose 44.0.

[b] Zn free mineral mixture (%): NaCl 5.0, Mg $SO_4 \cdot 7H_2O$ 74.5, $FeC_6H_5O_7 \cdot nH_2O$ 12.5, Zn-free mineral mix.[b*] 5.0, Cellulose 3.0 [b*] (%)— $AlCl_3 \cdot 6H_2O$ 10, $CoCl \cdot 6H_2O$ 1, KIO_3 3, Cellulose 986.

[c] The vitamin mix (%):-Thiamine hydrochloride 6, Riboflavin 10, Pyridoxine hydrochloride 4, Cyanocobalamin 0.01, Ascorbic acid 500, Niacin 40, Ca-pantothenate, 10, Inositol 200, Biotin 0.6, Folic acid 1.5, *p*-aminobenzoic acid 5, Vitamin K_3 5, Vitamin A acetate 4000 IU, Vitamin D_3 4000 IU.

Table 2. Proximate composition of the experimental diets (dry matter basis)

Parameters	Diets			
	P0Z0	P0Z1	P1Z0	P1Z1
Moisture (%)	4.5	4.5	3.0	4.5
Crude ash (%)	11.1	10.8	13.7	13.6
Crude protein (%)	44.9	44.7	45.5	45.2
Crude lipid (%)	16.6	16.7	16.6	16.9
Gross energy (kcal/g)	5.2	5.2	5.0	5.0

Table 3. Mineral contents of the experimental diets (dry matter basis)

Macro elements (mg/g)	Diets			
	P0Z0	P0Z1	P1Z0	P1Z1
P	19.14	19.49	30.30	30.09
Ca	28.03	30.19	34.76	35.20
Mg	3.00	3.05	3.00	2.97
Na	3.86	3.41	3.58	3.82
K	4.71	3.75	4.32	4.50
Trace elements (μg/g)				
Zn	54.54	103.2	55.85	96.07
Mn	40.44	40.71	41.18	40.62
Fe	345.5	341.9	352.2	348.2
Cu	7.04	7.05	10.63	8.35

Eyed eggs of rainbow trout were obtained from Fuji Trout Farm of Shizuoka Prefecture Fisheries Experiment Station and hatched under laboratory conditions at the Tokyo University Marine Science and Technology. Fish with an average body weight of 1.56 ± 0.24 g were randomly selected from stock and distributed into 60 L tanks at a density of 30 fish per tank. Duplicate groups were assigned to each experimental diet and the feeding was conducted for 21 weeks. The fish were hand fed three times per day, 6 days a week to apparent satiation level. The tanks had a continuous water supply at a rate of 0.6-1.0 l/min and the temperature was 15 to 24°C.

The fish were starved for 24 h before being individually weighed at the initial day and every 21 days of the experimental period after being anesthetized with ethylene glycol monophenyl ether (300 ppm). At the same time 5 fish were randomly sampled from each tank and stored at –20°C for analyses.

Proximate composition and chemical analysis of the diets and fish whole body samples were made in three replicates as follows: moisture contents was measured gravimetrically, crude ash contents was determined by incinerating a known amount of sample in an electric muffle furnace (Yamato, FA-21) at 600°C for 8 hours, crude protein was analyzed using the Kjeltec Auto Analyser System 1035/38 (Netherland), and crude lipid was measured by following the method of Folch *et al.* (1957). Samples for minerals were digested in nitric acid using the MLS-1200 Mega Microwave Digestion System (Italy), cooled in flowing water for 30 minutes, and diluted with de-ionized water to the required volume. Concentration of each element was measured by a Polarized Zeeman Atomic Absorption Spectrophotometer (Hitachi Z-5010, Tokyo, Japan) except for phosphorus which was analyzed by a visible light spectrophotometry (Shimadzu, UV 265 FW, Kyoto, Japan) at 750nm.

Statistical analyses of the results were performed using one-way and two-way ANOVA with SYSTAT 8.0 software (SPSS Inc. Chicago, USA, 1998). Differences between treatments were evaluated by Tukey's test. The level of significance was set at $p<0.05$ for all tests.

Results and discussion

In rainbow trout, weight gain of the fish did not show any significant different among treatments of both P and Zn throughout the culture period. Likewise insignificant differences were obtained among dietary Zn Level treatments; SGR (Specific growth rate), FCR (Feed conversion ratio) and TGC were not significantly affected by the treatment (Table 4). The results of growth performance and feed utilization indicate that both P and Zn regardless of their supplementing levels had no influence on the feed intake and growth performance of fish. In addition, the results represent the stated parameters not to be potential and appropriate indices to assess P and Zn levels. This is in agreement with Apines (2000) who stated weight gain as an inappropriate index of Zn bioavailability in rainbow trout. Similar results were obtained by Hardy and Shearer (1985) and Li and Robinson (1996).

Table 4. Growth and feed performance of the experimental diets for 21 weeks

Diet group	Weight gain	SGR[1] (% day^{-1})	FCR[2]	TGC[3]	Condition factor
P0Z0	73.62^{a}	2.62^{a}	0.97^{a}	0.001125^{a}	1.161^{a}
P0Z1	74.61^{a}	2.62^{a}	0.96^{a}	0.001118^{a}	1.153^{a}
P1Z0	68.88^{b}	2.50^{b}	1.01^{b}	0.001047^{b}	1.162^{a}
P1Z1	61.54^{b}	2.51^{b}	1.02^{b}	0.001021^{b}	1.125^{a}
P	<0.05	<0.05	<0.05	<0.05	NS
Zn	NS	NS	NS	NS	NS
P × Zn	NS	NS	<0.05	NS	NS

[1] Specific growth rate; [2] Feed conversion ratio. [3] Thermal-unit growth coefficient. NS = Not significant
* Values in the same column not sharing a common superscript letter are significantly different ($p<0.05$).

Carcass proximate composition of rainbow trout at start and end of the experiment (Table 5) revealed significant ($p<0.05$) influence of P supplementation level on whole body crude ash contents, whereas that of Zn supplementation on crude ash was not remarkable.

Table 5. Proximate carcass composition of fish at start (n=30) and end (n=12) of the experiment

Diet group	Moisture (%)	Crude ash (%)	Crude protein (%)	Crude lipid (%)
P0Z0	67.37	1.97^{b}	16.46	14.35
P0Z1	67.33	2.03^{ab}	16.26	14.33
P1Z0	68.58	2.36^{a}	16.28	12.71
P1Z1	68.58	2.17^{ab}	16.31	13.22
P	NS	<0.05	NS	NS
Zn	NS	NS	NS	NS
P × Zn	NS	NS	NS	NS

Values in the same column not sharing a common superscript letter are significantly different ($p<0.05$).
NS = Not significant.

Insignificant variations were observed among different treatments of both P and Zn in the cases of whole body P, Ca, Mg, Na, K and Mn contents (Table 6). Conversely, whole body Zn content was found to increase significantly ($p<0.05$) in the treatments with higher Zn supplementation (Table 6). Supplementation of P also showed significant ($p<0.05$) increase in the whole body Fe content. Higher whole body Zn content with dietary Zn supplementation in this study is in agreement with the other study in Atlantic Salmon (Maage and Julshamn 1993). Zinc concentration of Abalone also increased linearly with dietary Zn (Tan and Mai 2001).

Table 6. Whole body mineral contents of fish at start (n=30) and end (n=12) of the experiment

Initial/ Diet group	P (mg/g)	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Zn (μg/g)	Fe (μg/g)	Cu (μg/g)	Mn (μg/ g)
P0Z0	4.43	3.94	0.33	0.7	3.56	15.2^{ab}	9.61	1.00	1.36
P0Z1	4.29	3.48	0.31	0.67	3.73	23.5^{a}	9.01	1.11	0.99
P1Z0	4.98	4.87	0.35	0.72	3.65	13.7^{b}	11.5	1.25	1.16
P1Z1	4.49	3.89	0.32	0.64	3.63	21.9^{a}	10.3	1.04	1.11
P level	NS	NS	NS	NS	NS	NS	<0.05	NS	NS
Zn level	NS	NS	NS	NS	NS	<0.05	NS	NS	NS
P level × Zn level	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values in the same column not sharing a common superscript letter are significantly different ($P<0.05$). NS = Not significant.

Bone mineral contents of the fish are presented in the Table 7 and Figure 1. Dietary Zn and P supplementation significantly ($p<0.05$) influenced vertebral Zn and Fe contents in rainbow trout. Bone Zn content was significantly ($p<0.05$) affected by the dietary levels of P and Zn (Fig. 1). In addition, the interaction between P and Zn levels were also observed to have influence on Mg and Fe concentration of bone. Higher bone Zn accumulation obtained in treatments with higher supplementation for rainbow trout was similar to the findings of Satoh *et al.* (1987). In channel catfish, bone Zn increased linearly as $ZnSO_4$ and ZnMet increased (Li and Robinson 1996). Mohanna and Nys (1999) had similar results with chickens where tibia Zn concentration increased linearly with dietary Zn content. Zinc concentration of tissues of rats dependent on the dietary Zn levels (Roth and Kirchgessner 1983). The present results indicated that highest bone deposition was achieved with higher Zn supplemented diets. Zinc content in bones was higher than whole body probably because after absorption in the digestive tract, Zn was absorbed in the skeletal tissues (Knox *et al.* 1982) where it was deposited. Moreover, the skeleton acts as a reservoir for Zn (Yamaguchi 1998). The incorporation of Zn in the skeleton is relatively slow and it is firmly bound for long periods. When bones are already saturated, the excess amount of Zn is stored in the skin and muscle (Knox *et al.* 1982). Jeng and Sun (1981) similarly observed that Zn levels increased in the skeletal

tissues of common carp tissue then it deposits in the muscle tissues when fed high levels of $ZnSO_4$. Bone Zn has also been shown to be a more sensitive criterion of Zn status than weight gain in various animals (Gatlin and Wilson 1983, Forbes *et al.* 1984). Previous studies also indicated that whole body (Wekell *et al.* 1986) and bone levels (Huber and Gershoff 1970) have been used to quantify Zn status in animals. As in higher vertebrates, these results indicate that different tissues have varying rates of elemental deposition in fish.

Table 7. Vertebral mineral contents of fish at the end of the experiment (n=12) fed experimental diets for 21 weeks

Diet group	P (mg/g)	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Fe (μg/g)	Cu (μg/g)	Mn (μg/g)
P0Z0	112.57	206.14^{c}	4.22^{ab}	3.45	3.88	17.82^{a}	22.20	37.99
P0Z1	115.48	2.08.33^{bc}	4.12^{ab}	3.28	3.54	17.85^{a}	20.19	38.11
P1Z0	116.30	210.84^{ab}	4.78^{a}	3.48^{b}	4.01	16.44^{b}	32.24	35.51
P1Z1	116.86	212.76^{a}	3.76^{b}	3.73	4.21	10.24^{c}	11.44	40.69
P	NS	<0.05	NS	NS	NS	<0.05	NS	NS
Zn	NS	<0.05	<0.05	NS	NS	<0.05	NS	NS
P × Zn	NS	NS	<0.05	NS	NS	<0.05	NS	NS

Values in the same column not sharing a common superscript letter are significantly different (P<0.05). NS = Not significant.

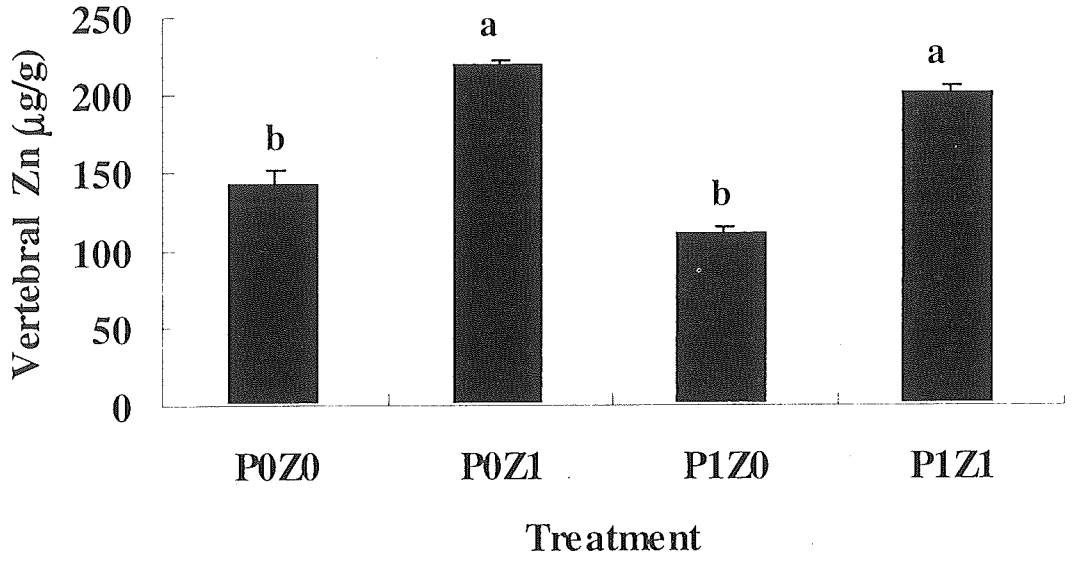

Fig. 1. Effect of dietary P and Zn levels on vertebral zinc contents of rainbow trout at the end of the experiment (n=12) fed experimental diets for 21 weeks.

The overall results of the present study demonstrated that P supplementation is not needed in the diet of fingerling rainbow trout for growth and bone mineralization. On the other hand Zn supplementation is needed. Yet, further studies in this area with different size and age groups of rainbow trout are warranted.

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